Impact of Various Types of Genetic Damage and Risk Assessment

by James F. Crow*

I should like to review very briefly the various kinds of genetic damage that might be expected to occur from chemical mutagens, the time delay before such effects might be manifest, and something of the kind of impact that these might be expected to have on human well-being. I hardly need to add that this is an area where we know very little; we know enough to be apprehensive, but not enough to be at all certain.

My remarks will draw heavily from two recent reports on radiation effects, the National Academy of Sciences Report (*1*) and the United Nations Report (*2*). In the area of radiation protection there are standards which are agreed to internationally and which form the basis of radiation protection policy. I suggest that in the area of chemical mutagenesis we can obtain considerable guidance from the older field of radiation mutagenesis. I shall have a specific suggestion later as to how radiation standards can be used as a starting point in setting limits for chemical mutagens.

What Kinds of Effects Might Mutagens Produce?

For assessment of the genetic risk to the population, it is convenient to classify genetic damage to man under four very general headings.

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Mendelian

With increasing knowledge we will be able to distinguish between the various kinds of molecular changes in the gene and minute chromosome aberrations, but for most human traits we must now be content to group together all those that are inherited as mendelian units. Within this class we can divide them meaningfully into autosomal dominant, autosomal recessive, and X-linked recessive. Of course, there are X-linked dominants and perhaps a few Y-linked traits, but these are numerically insignificant.

In his recent compedium, McKusick (*3*) lists 415 dominantly inherited traits and an additional 528 if less well-established conditions are included. In most cases the homozygous phenotype of these genes is unknown; they are called dominant because the heterozygous expression is sufficient to cause a recognizable abnormality or disease. There are 86 X-linked traits listed plus 64 more if less certain types are included. Most of these are recessive, and therefore the abnormality is mainly in males. He lists 365 well-established autosomal recessive traits and 418 more that are probably inherited this way.

In well studied organisms such as *Drosophila* and mouse, the ratio of recessive to dominant mutants is much higher than it is in man. Undoubtedly this means that many, probably the great majority, of recessive mutants in man have not yet been discovered. Unless there is consanguinity of the parents, or multiple occurrences within a sibship, or

a particularly characteristic phenotype, recessive inheritance is often very difficult to identify.

Cytogenetic Abnormalities

These can be subdivided into two broad categories: (a) those caused by errors in the distribution of chromosomes leading to abnormalities of chromosome number and (b) the consequences of chromosome breakage. The latter often lead to unbalanced chromosome combinations. In either case, the usual consequences are various abnormalities, physical and mental, and embryonic death.

Complex Inheritance

There are a number of abnormalities and diseases for which there is strong evidence that genetic factors are important, but which do not follow any recognizable mendelian pattern. This may be caused by irregularity of expression of a single gene (incomplete or variable penetrance) or the effect may be the consequence of multiple genes. Many congenital anomalies belong to this category, as do a number of constitutional and degenerative diseases.

Minor Effects

We know almost nothing about this category in man or other mammals, but in *Drosophila* the most frequent kind of mutational event is a mutant whose effects are too small to be detected in the individual fly, but which cause a statistically detectable decrease in the probability of survival to adulthood, or in fertility.

The incidence of these per million live births, as given in the BEIR report (*1*), is summarized in Table 1.

The category, "constitutional and degenerative diseases," is admittedly very uncertain. This number can be made to have almost any value by the appropriate choice of which diseases to include (who is free of *all* physical imperfections?). The number given includes most of the severe conditions thought to have some genetic basis and which are expressed in childhood or early adulthood.

Table 1.

	Incidence, 10^{-6} live births	
Mendelian		
Autosomal dominant	10,000	
X-linked	400	
Autosomal recessive	1,500	
		12,000
Cytological congenital anomalies		
Aneuploidy	4,000	
Unbalanced rearrangements	1,000	
		5,000
Complex inheritance		
Congenital anomalies	15,000	
Anomalies expressed later	10,000	
Constitutional and degenerative diseases	15,000+	
		40,000
		60,000
Abortion (recognized)		
Aneuploidy	35,000	
XO	9,000	
Unbalanced rearrangements	11,000	
		55,000
Minor and polygenic		? ? ?

Degree of Dependence of Incidence on Mutation Rate

Mendelian dominant genes that cause moderate to severe disease are usually new mutations only a few generations old. In general, the more severe the disease, the shorter time the gene persists in the population and the greater the fraction of mutant genes that are new. For dominantly inherited traits, the incidence of the disease is, for all practical purposes, simply proportional to the mutation rate.

How soon would the increase occur? Dominant mutants are expressed in the generation immediately following their occurrence (unless there are complications, such as reduced penetrance). If the mutation rate were, say, doubled, there would be a doubling of that fraction of the cases which are new mutants. If the mutation rate were to remain at the doubled rate, the incidence would rise until eventually the total incidence became twice as high. The time required for this state to be reached would depend roughly on how harmful the gene is. The milder the effect, the more gradual the rise.

The story is somewhat similar with X-linked recessive genes, except that only about one-third of the mutant genes are expressed in any particular generation. Thus the impact is only one-third as large the first generation and rises to its final value at a slower rate.

The effect of recessive gene mutations is likely to be delayed for many generations, perhaps hundreds. The gene will become homozygous either if it happens to be inherited by the same person that receives a similar preexisting gene from the other parent, or if two genes each descended from the original mutant get into the same individual by way of his father and mother (who must therefore be related). The low pre-existing frequency, in the one case, and the low incidence of consanguineous marriage, in the other, collectively insure that homozygosity for a recessive mutant has a very low probability. It is thus delayed for a very long average time. Even more likely, I suspect, is that the mutant never becomes homozygous; it is likely to be eliminated from the population through minor effects on viability and fertility in the heterozyous condition. At least this is what we would expect by analogy with what happens in *Drosophila*.

With chromosome abnormalities most of the damage occurs soon after the cause. If there is an increase in nondisjunction or chromosome loss following some environmental chemical, the effect will begin the next generation, and virtually the whole effect will be in that one generation. This is because almost all aneuploidy leads to embryonic death, early postnatal death, or to some condition that reduces the net fertility to essentially zero.

Unbalanced rearrangements caused by chromosome breakage may be delayed by several generations, but most of the impact of the genetic damage is within the first half-dozen generations.

For more complexly inherited traits it is almost a pure guess, both as to the extent and the time of expression following the chemical influence.

I have been very sketchy in this review. More details are available in the National Academy report (*1*) and especially in the United Nations report (*2*).

The area of greatest uncertainty is minor, quantitative effects. As I said earlier, in well studied organisms, especially *Drosophilia*, the most common known mutant type is one causing a minor effect on viability. One might think that mutants that are too mild to have a noticeable effect can be ignored. But there are two arguments in opposition to this view. One is that what is not noticeable in *Drosophila* may be very important to a human being. (You can't ask a fly where it hurts.) Also, the milder the effect the mutant has, the less is its probable effect on fertility; and therefore, the longer it will persist in the population. By affecting a larger number of persons to a mild degree, the overall impact of the mutant may be as great as if it affected a smaller number more severely. This becomes especially important if there are many such mutants, so that each person is afflicted with several; no one may mean much, but their cumulative effect may mean an appreciable impairment of some body function.

Most attempts to quantitate the effect of a mutation increase in man ignore this category. Since I have no idea how to quantify it, I will, too—but with the uneasiness that comes from the fear that we may well be ignoring the most important part.

It is clear that we have almost no quantitative information on how an increased mutation rate, whatever its cause, would affect human survival, health, and well-being. It is also clear that we could have a substantial increase in the mutation rate without knowing it. We are reasonably agreed that the effect of any increase in the mutation rate would be harmful, but how harmful?

What Do We Do When a Chemical Is Found to be Mutagenic in a Test System?

The answer to this question is simple if: (a) the substance has no benefits, or (b) a tested, harmless substitute is available. In these cases, few would argue for the continued use of the substance.

Alternatively, a compound that is used exclusively for the treatment of a severe disease that is rare or that affects mainly people over 40 years of age might be highly mutagenic, but few would argue against its use if it were of great benefit.

What do we do if the chemical has a definite benefit to many and is found to be mutagenic? I think we have to have some sort of quantitative criterion. Ultimately we must try to balance benefit against risk, as is tried for x-rays and nuclear energy.

First we need some kind of estimate of the dose to the population. We need to know: (a) how large a fraction of the population would be exposed (b) the dosage (of course we are interested in the dose that reaches the reproductive cells), (c) the age and sex distribution of the dose. We would then try to compute something like the average genetically significant dose (GSD) to the population, as is done for radiation. By this we mean the gonad dose weighted by the expected number of children to be born. To do this we need to know age and sex-specific survival and fertility rates.

Given this information, it will still be some time before we are able to make any reasonable estimate of the risk. What do we do in the meantime? I suggest that we use radiation as a guideline.

Chemical Mutagens Assessed in Terms of Radiation Equivalents

How has the question been dealt with for radiation? In general there is an attempt to balance the cost against the benefit. Even though both factors to be weighed are very uncertain, it may be that the benefit is so great or so minute that even a very rough calculation of risk may suffice for a rational decision.

The most reliable criterion for setting radiation standards for the general population has been the background level of radiation. I think that despite numerous attempts to quantify the genetic risk, the most convincing argument we have the for the present radiation standards is that they are close to the natural background radiation.

The present standard for the general population is 170 mrem per year from all nonmedical, man-made causes. The average background radiation is about 100 mrem per year. It is generally believed, although the evidence is far from complete, that natural radiation accounts for only a minor fraction of the total human mutation rate. Therefore a doubling of the amount of radiation would double only that fraction of the mutation rate that is caused by natural radiation. Man has survived background radiation throughout his entire history and, although there is no reason to think that it has been good for him, the harm done by it has been something that he has been able to tolerate.

Therefore I suggest that we try to tie chemical mutagenesis in with radiation. I suggest that chemical mutagens be assessed in terms of roentgen equivalents, or a roentgen equivalent dose (*RED*). [I am pleased to see that Dr. Bridges (*4*) has independently arrived at the same idea. He suggests the word radeq, for radequivalent.]

Even if we not know how to measure the impact on man for either radiation or chemicals (and for the most part we do not), we can at least try to keep the level of chemical mutagens in the environment at a level such that their effect as determined in test systems is no greater than that produced by radiation at a level equivalent to that which occurs spontaneously.

If a chemical of obvious benefit is developed, we can then keep it at a level such that the genetically significant dose to the population per year is less than the maximum permissible radiation dose of 170 mrem in terms of the amount of damage caused in the test system. If several chemicals get into the environment, it is necessary to keep their total GSD less than the equivalent of 170 mrem.

I realize that there are both uncertainties and complications. The major uncertainty is whether the relative effectiveness of chemicals and radiation in the test system is the same as in man. We cannot be sure, but we

can get steady improvement in our confidence by using several test systems for the most important chemicals (those to which the largest number of persons are exposed), and choosing systems as close to man as possible. We are almost certain to find that the chemical/radiation ratio of effectiveness will differ with different endpoints—say, chromosome breakage, versus base substitutions. Perhaps the criterion should then be the prudent one of using the criterion that is safest, i.e., that maximizes the risk estimate.

If we knew more, we could try to set environmental chemical standards so that the induced mutation rate would be less than some fraction (say, 10% of the spontaneous mutation rate. That is, we would choose a concentration that in 30 years of exposure would produce a certain fraction of the spontaneous human mutation rate. However, we cannot make such a calculation now, for we know neither the human spontaneous mutation rate nor the effect of the mutagen.

So this kind of assessment cannot now be made yet. Until it can be, I suggest the radiation-equivalent concept as the best way of getting started in dealing with setting standards for chemicals that are slightly mutagenic, but otherwise of sufficient benefit that this appears to outweigh the risk.

I should like to end by acknowledging the help I have gotten from Dr. Seymour Abrahamson. Several of the ideas presented here are the result of discussions with him.

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